

Extended Stability of Rectal Swab Specimens for Carbapenemase Gene Detection Using the Cepheid Xpert® Carba- R Assay

S. Morris| K. Cummings, M.Y. Khan, A Marcinkiewicz, N Faraci, J. Bodnar, C; Jacobsen, K. Miller, S. Kennedy, M. Foster, E. Nazarian, K.A. Musser
Wadsworth Center, NYSDOH, Albany, NY



Department of Health
Wadsworth Center

BACKGROUND

- The Wadsworth Center (WC) serves as the Northeast Regional Antimicrobial Resistance (AR) Laboratory, specializing in the identification and characterization of carbapenemase-producing organisms (CPOs).
- WC performs colonization screenings (CS) from rectal swabs using the FDA-cleared Cepheid Xpert® Carba-R PCR assay, which detects five major carbapenemase genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, and *bla*_{IMP}).
- This CS is performed in collaboration with healthcare facilities, infection preventionists, and epidemiologists to screen high-risk patients and assess CPO transmission events.
- According to the FDA cleared package insert, rectal swabs collected are stable for up to five days at 15 °C to 28 °C prior to testing.
- WC conducted stability studies to evaluate extended storage and transport times and temperatures, as well as recovery of viable gene-positive organisms.

METHODS

Mock rectal swab specimens were prepared by inoculating sterile COPAN Swabs with pooled, known-negative fecal samples. 200 dual rectal swab collections kits (400 swabs in total) were spiked for this study, with the first swab being used for PCR and the second swab for culture, isolation and target gene confirmation.

Inoculated fecal swabs were dosed with 50 µL of a bacterial suspension containing a target carbapenemase gene, *bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, or *bla*_{IMP}. (Table 1.)

| Carbapenemase Gene | Organism | Carbapenemase Gene Variant | Concentration |
|------------------------------|--------------------------------|------------------------------|---------------|
| <i>bla</i> _{KPC} | <i>Escherichia coli</i> | <i>bla</i> _{KPC-3} | 10-3 |
| <i>bla</i> _{NDM} | <i>Acinetobacter baumannii</i> | <i>bla</i> _{NDM-1} | 10-3 |
| <i>bla</i> _{OXA-48} | <i>Klebsiella pneumoniae</i> | <i>bla</i> _{OXA-48} | 10-3 |
| <i>bla</i> _{VIM} | <i>Enterobacter cloacae</i> | <i>bla</i> _{VIM-1} | 10-2 |
| <i>bla</i> _{IMP} | <i>Pseudomonas aeruginosa</i> | <i>bla</i> _{IMP-1} | 10-3 |

Table 1.

The inoculated swabs were stored at various temperatures across multiple time points (Days 0, 1, 4, 6, 8, 11, 15, 21, 28, and 46/47) to mimic colonization screening transport conditions. (Table 2.)

| Temperature Ranges During Study |
|--------------------------------------|
| Freezer: -18 °C to -20 °C |
| Refrigerated: 3.5 °C to 4 °C |
| Room Temperature: 19.0 °C to 22.8 °C |
| Incubated: 35.5 °C |

Table 2.

At the specific time point, PCR testing was performed with the Carba-R assay on the GeneXpert® Infinity System. For positive PCR samples the cycle threshold (CT) was recorded, and the second swab was cultured on CHROMagar™ mSuperCARBA™ plates and evaluated for organism/gene recovery. Isolated colonies were then tested with the NG-Test® CARBA-5 for confirmation of the target carbapenemase. (Figure 1.)

WORKFLOW

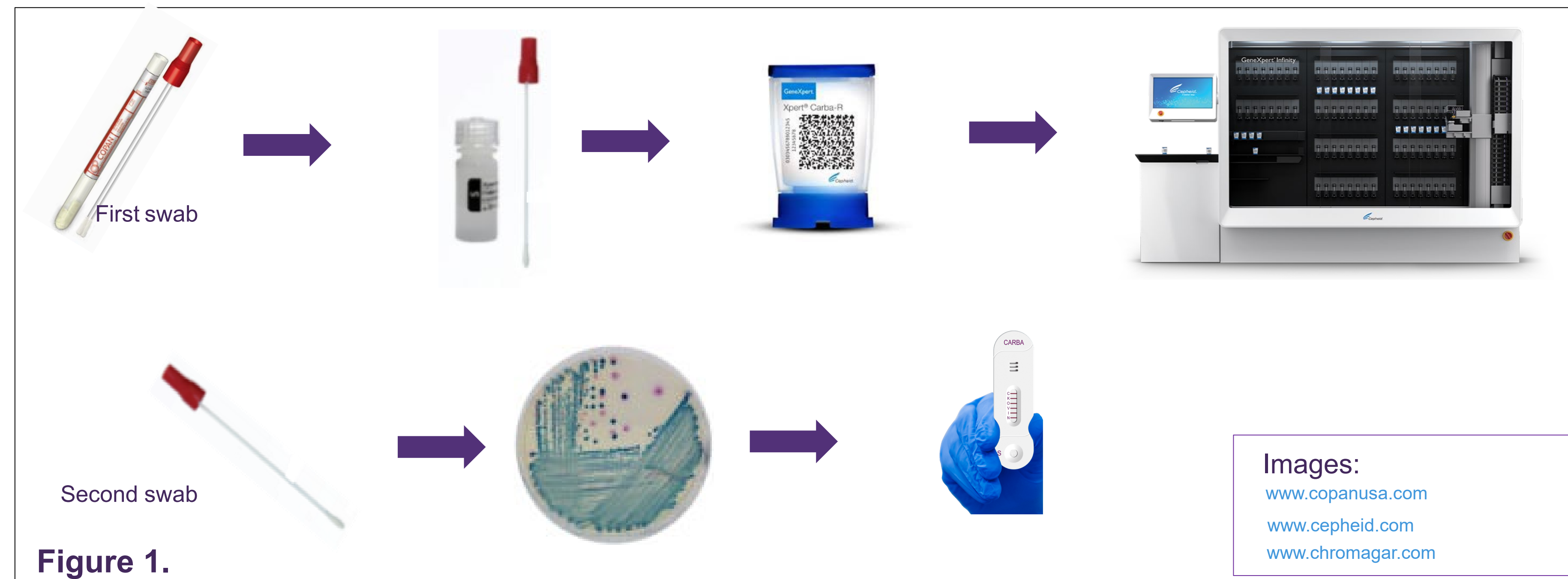


Figure 1.

Cepheid Xpert® Carba-R PCR RESULTS

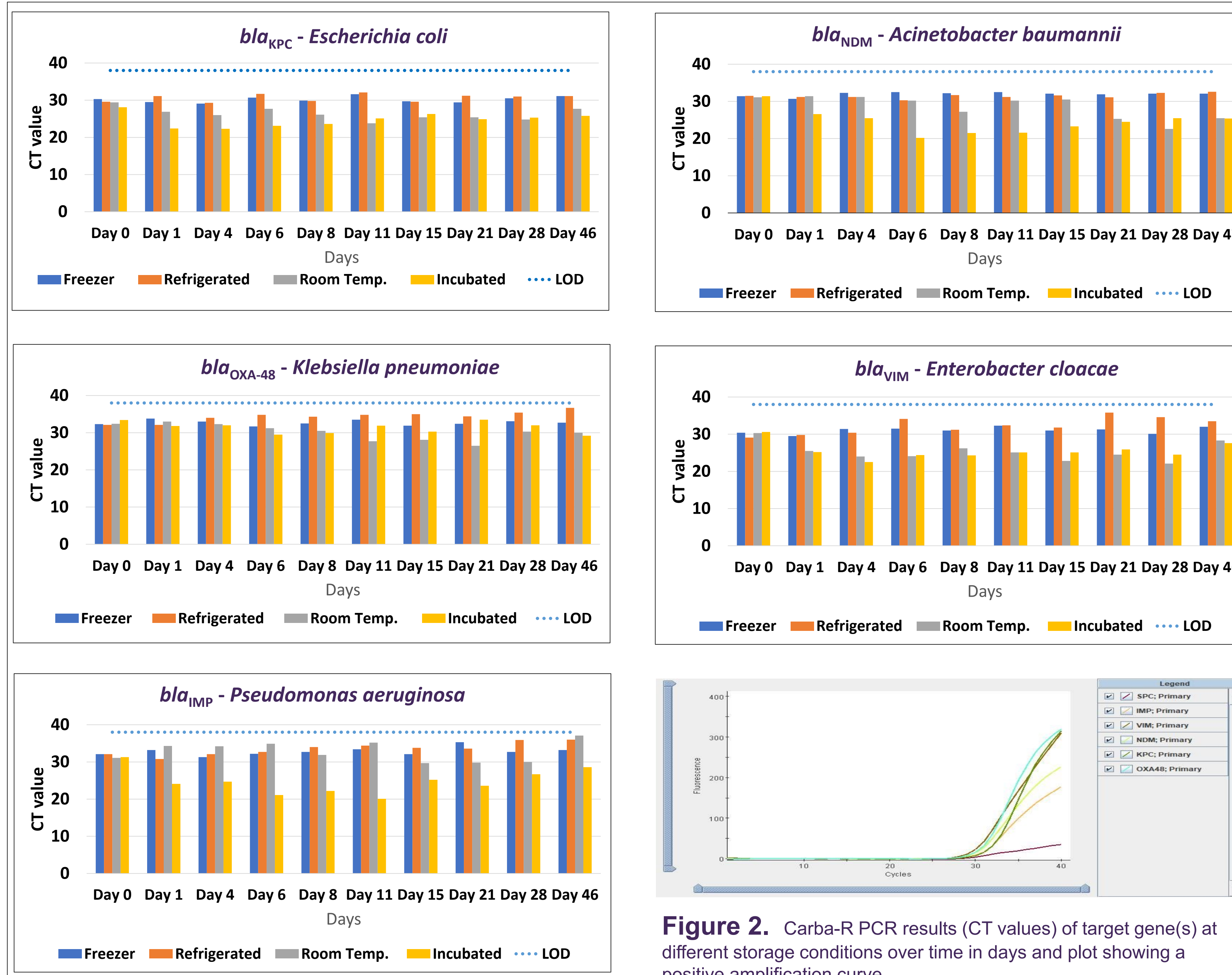


Figure 2. Carba-R PCR results (CT values) of target gene(s) at different storage conditions over time in days and plot showing a positive amplification curve.

CULTURE RESULTS

| Storage Conditions | Storage Time (Days) | | | | | | | | | | |
|--------------------|---------------------|---|---|---|---|----|----|----|----|----|---|
| | 0 | 1 | 4 | 6 | 8 | 11 | 15 | 21 | 28 | 46 | |
| Freezer | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Refrigerated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Room Temperature | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Incubated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |

Table 3. Culture growth results of *Escherichia coli* containing the *bla*_{KPC} gene after rectal swab storage.

| Storage Conditions | Storage Time (Days) | | | | | | | | | | |
|--------------------|---------------------|---|---|---|---|----|----|----|----|----|--|
| | 0 | 1 | 4 | 6 | 8 | 11 | 15 | 21 | 28 | 46 | |
| Freezer | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Refrigerated | Y | Y | Y | Y | Y | Y | Y | Y | Y | N | |
| Room Temperature | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Incubated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |

Table 4. Culture growth results of *Acinetobacter baumannii* containing the *bla*_{NDM} gene after rectal swab storage.

| Storage Conditions | Storage Time (Days) | | | | | | | | | | |
|--------------------|---------------------|---|---|---|---|----|----|----|----|----|--|
| | 0 | 1 | 4 | 6 | 8 | 11 | 15 | 21 | 28 | 46 | |
| Freezer | Y | Y | Y | Y | Y | Y | Y | Y | Y | N | |
| Refrigerated | Y | Y | Y | Y | Y | Y | Y | N | Y | N | |
| Room Temperature | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Incubated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |

Table 5. Culture growth results of *Klebsiella pneumoniae* containing the *bla*_{OXA-48} gene after rectal swab storage.

| Storage Conditions | Storage Time (Days) | | | | | | | | | | |
|--------------------|---------------------|---|---|---|---|----|----|----|----|----|--|
| | 0 | 1 | 4 | 6 | 8 | 11 | 15 | 21 | 28 | 46 | |
| Freezer | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Refrigerated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Room Temperature | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Incubated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |

Table 6. Culture growth results of *Enterobacter cloacae* containing the *bla*_{VIM} gene after rectal swab storage.

| Storage Conditions | Storage Time (Days) | | | | | | | | | | |
|--------------------|---------------------|---|---|---|---|----|----|----|----|----|--|
| | 0 | 1 | 4 | 6 | 8 | 11 | 15 | 21 | 28 | 46 | |
| Freezer | Y | Y | Y | Y | N | N | Y | N | N | N | |
| Refrigerated | Y | Y | Y | Y | Y | Y | N | N | Y | N | |
| Room Temperature | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |
| Incubated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | |

Table 7. Culture growth results of *Pseudomonas aeruginosa* containing the *bla*_{IMP} gene after rectal swab storage.

| Storage Conditions | 0 | 1 | 4 | 6 | 8 | 11 | 15 | 21 | 28 | 46 |
|--------------------|---|---|---|---|---|----|----|----|----|----|
| Freezer | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Refrigerated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Room Temperature | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Incubated | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |

Tables 3. - 7.

CONCLUSIONS

- Stability studies demonstrated that bacterial DNA on rectal swabs collected using COPAN Dual Swab Sample Collection Device and tested using the Xpert® Carba-R assay were stable at all temperatures from -18 °C to 35.5 °C. In addition, the rectal swabs were positive for all target genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{OXA-48}, and *bla*_{IMP}) for at least 46 days post collection.
- Culture of Carba-R positive swabs demonstrated organism/gene recovery was optimal at room temperature or incubated for up to 46 days. The percent recovery was 100% at these conditions. Samples that were stored refrigerated or frozen had a recovery rate of 94%. Viability varied and was determined to be 6 days when stored frozen, 11 days when refrigerated, and 46 days at room temperature or incubated at 35.5 °C.
- These findings demonstrate that rectal swabs collected and tested with the Xpert® Carba-R PCR assay maintain stability and gene detection well beyond the current FDA-cleared specifications. This study will allow our laboratory to extend acceptability criteria and will provide the ability to perform testing when exceptional circumstances delay shipments to continue to provide public health impact by identifying and stopping CPO transmission in healthcare facilities.

ACKNOWLEDGMENTS

This work was supported by the New York State Department of Health, Cooperative Agreement Number NU50CK000423 funded by the Centers for Disease Control and Prevention (CDC), Cooperative Agreement Number U60OE000103 funded by the CDC through the Association of Public Health Laboratories.